

## ESTIMATING GENETIC VARIATION IN THE BLACK-FOOTED FERRET— A FIRST ATTEMPT

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**ABSTRACT.**—No genetic variation was observed for three proteins examined from samples of saliva from 22 black-footed ferrets (*Mustela nigripes*). The comparable data concerning levels of genetic variation in other taxa at these loci are too inconclusive to provide a meaningful interpretation of the observed absence of genetic variation. The absence of genetic variation observed in the black-footed ferret population is compatible with the reported levels of genetic variation in terrestrial carnivores and populations that have undergone bottlenecks. Suggestions for additional studies using different approaches both to increase the number of loci that are used to determine the level of genetic variability in the black-footed ferret and to provide a more meaningful comparative data base are provided.

The importance of genetics in the management and conservation of endangered species has been recently discussed (Soulé and Wilcox 1980, Frankel and Soulé 1981, Schonewald-Cox et al. 1983). Although the primary objective of conservation and management is the continued reproduction of the species, maintenance of genetic variability has also been identified as a high priority (Benirschke 1977, Chesser et al. 1980). Without maintenance of genetic variability, the species may have an increased probability of extinction in future variable environments (Wright 1951).

The objective of this study was to obtain an estimate of the level of genetic variability present in a population of the endangered black-footed ferret (BFF) near Meeteetse, Wyoming. Salivary samples were easily taken and did not affect the survivorship of individuals sampled. Comparison of the genetic variation observed in the Meeteetse population with reported values in the literature on other species were made to determine the potential effect of the recent history of population size (bottlenecks) and isolation.

### METHODS

Salivary samples were collected from immobilized animals in the field during 1982 and 1983 by swabbing the oral and buccal cavities with a cotton swab or a small piece of gauze.

Samples were frozen and shipped on dry ice to the University of Vermont for analysis of electrophoretic variation of salivary proteins.

Salivary proteins were washed from the cotton or gauze with 1–2 ml of distilled water. The residual protein solution was removed from the cotton or gauze by centrifugation at 600–800 rpm. A corner of the cotton or gauze was held outside a 15-ml screw cap centrifuge tube before placing the cap on the tube to separate the cotton or gauze from the liquid during centrifugation. Samples were frozen at -75 C until analysis.

Prior to electrophoresis, salivary samples were concentrated by the use of acrylamide sticks (Curtain 1964, Balakrishnan and Ashton 1974). Salivary amylase (AMY) was examined by the methods of Aquadro and Patton (1980), except the sample was increased to 25  $\mu$ l per slot and the gel with the starch overlay was incubated overnight at 37 C before staining. Salivary esterase (EST-S) was examined by the methods of Tan (1976) except n-propanol was deleted from the stain. The method of Tan and Teng (1979) was usable for superoxide dismutase (SOD). Better results were obtained with a 10% acrylamide gel using the 8.9 tris-borate-EDTA buffer system of Coyne and Felton (1977) and with a stain of 100 ml of the SOD incubation buffer (Tan and Teng 1979), 30 mg MTT, 30 mg nitro blue tetrazolium, and 2 mg phenazine methosulfate. In addition, the methods of Tan and Ashton (1976a)

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TABLE 1. Genetic variability (heterozygosity) among terrestrial mammals in which two or more of the proteins examined from the saliva of black-footed ferrets have been reported. References in parentheses are cited below; data for *Herpestes auropunctatus* are unpublished (D. B. Hoagland, personal communication).

| Taxa                           | AMY      | EST      | SOD         | H*    | H**                 |
|--------------------------------|----------|----------|-------------|-------|---------------------|
| <i>Homo sapiens</i>            | 0.138(1) | 0.495(4) | 0.009(5)    | 0.214 | 0.067(12)           |
| <i>Peromyscus maniculatus</i>  | 0.315(2) |          | 0.000(6,7)  | 0.158 | 0.122(6,7,13,14,15) |
| <i>Peromyscus leucopus</i>     | 0.185(2) |          | 0.241(8)    | 0.213 | 0.081(7,8,16,17)    |
| <i>Mus musculus</i>            | 0.048(3) |          | 0.107(9,10) | 0.052 | 0.098(9,10)         |
| <i>Herpestes auropunctatus</i> | 0.000    | 0.000    | 0.000       | 0.000 | 0.037               |
| <i>Mustela nigripes</i>        | 0.000    | 0.000    | 0.000       | 0.000 | ?                   |
| h                              |          |          | 0.020(11)   |       |                     |

References: (1) Merritt et al. 1973, (2) Aquadro and Patton 1980, (3) Nielsen and Sick 1975, (4) Tan 1976, (5) Beckman and Pakarinen 1973, (6) Avise et al. 1979, (7) Loudenslager 1978, (8) Price and Kennedy 1980, (9) Selander and Yang 1969, (10) Selander et al. 1969, (11) Selander 1976, (12) Harris and Hopkinson 1972, (13) Dubach 1975, (14) Aquadro and Kilpatrick 1981, (15) Smith 1981, (16) Selander et al. 1975, (17) Browne 1977.

h = mean heterozygosity of locus.

H\* = mean heterozygosity of species based on loci examined in the black-footed ferret.

H\*\* = mean heterozygosity of species.

for salivary acid phosphatase, Tan and Ashton (1976b) for hexose-6-phosphate dehydrogenase, and Tan and Teng (1979) for lactate dehydrogenase, and saliva oxidase were attempted. No reactions were observed in the BFF salivary samples with these methods.

## RESULTS

No electrophoretic variation was observed among salivary samples from 22 BFFs at the loci for salivary amylase, salivary esterase, or superoxide dismutase. Based on this very small sample of loci ( $n=3$ ), the mean proportion of loci polymorphic ( $\bar{P}$ ) was 0.000 and the mean heterozygosity ( $\bar{H}$ ) was 0.000.

## DISCUSSION

Before the absence of genetic variability observed in the proteins from saliva of the BFF may be interpreted, some comparisons are needed. Most estimates of genetic variation are based on proteins from blood, liver, kidney, and heart or other muscle; the levels of genetic variability present in loci for salivary proteins are not well known. Saliva has been examined in very few taxa, and, in most cases, only salivary amylase has been analyzed. Superoxide dismutase, which was examined from saliva from the BFF, is also expressed in the more conventional tissue sources of proteins for electrophoretic analysis and is the only locus of the three analyzed that has been examined in a large number of taxa.

The extent of genetic variation at the three loci (AMY-1, EST-S, SOD-1) examined in the

BFF was estimated in other taxa by the methods of Lewontin and Hubby (1966) and Nei (1978). Few taxa could be found in the literature for which two or more of the loci had been examined for electrophoretic variation (Table 1). Only the locus for superoxide dismutase has been examined in a sufficient number of taxa of mammals to yield a reasonable estimate of the average amount of genetic variation present. Selander (1976) reported that SOD demonstrated a low mean heterozygosity ( $\bar{H} = 0.020$ ) among 26 species of rodents.

Although the loci for nonspecific esterases are generally considered highly variable, salivary esterase variation (Table 1) has only been reported in humans (Tan 1976). This locus apparently has not been examined in other taxa, although it is highly variable in humans.

Salivary amylase has typically not been included among the proteins examined in surveys of genetic variation in mammals. The taxa of mammals for which estimates of heterozygosity are available or from which estimates can be calculated (Merritt et al. 1973, Nielsen and Sick 1975, Aquadro and Patton 1980) appear to represent taxa in which this locus is highly variable (Table 1).

Considerable variation in levels of genetic heterozygosity at the three loci was observed, ranging from high genetic heterozygosity in *Peromyscus* to no heterozygosity in *Herpestes* (Table 1). The estimates of average heterozygosity calculated from three loci (AMY-1, EST-S, SOD-1) (Table 1) are greater than the reported heterozygosity calculated from a greater number of loci in man and *Peromyscus* but lower in *Mus* and *Herpestes* (Table 1).

TABLE 2. Genetic variation among terrestrial carnivores.

| Taxa                           | Sample size |      | Mean proportion of loci    |                             | Reference             |
|--------------------------------|-------------|------|----------------------------|-----------------------------|-----------------------|
|                                | Individuals | Loci | Polymorphic per population | Heterozygous per individual |                       |
| Canidae                        |             |      |                            |                             |                       |
| <i>Canis lupus</i>             | 12          | 53   | 0.113                      | 0.030                       | Fisher et al. 1976    |
| <i>Canis latrans</i>           | 6           | 53   | 0.132                      | 0.050                       | Fisher et al. 1976    |
| <i>Canis familiaris dingo</i>  | 6           | 53   | 0.057                      | 0.006                       | Fisher et al. 1976    |
| <i>Vulpes vulpes</i>           | 282         | 21   | 0.000                      | 0.000                       | Simonsen 1982         |
| Ursidae                        |             |      |                            |                             |                       |
| <i>Ursus americanus</i>        | 56          | 6    | 0.000                      | 0.000                       | Manlove et al. 1980   |
|                                | 35          | 14   | 0.077                      | 0.013                       | Manlove et al. 1980   |
|                                | 64          | 15   | 0.133                      | 0.015                       | Manlove et al. 1980   |
|                                | 52          | 17   | 0.176                      | 0.031                       | Manlove et al. 1980   |
|                                | 52*         | 33   | 0.121                      | 0.016                       | Manlove et al. 1980   |
|                                |             |      | $\bar{x} = 0.097$          | $\bar{x} = 0.015$           | Manlove et al. 1980   |
| <i>Ursus maritimus</i>         | 52          | 13   | 0.000                      | 0.000                       | Allendorf et al. 1979 |
| Procyonidae                    |             |      |                            |                             |                       |
| <i>Procyon lotor</i>           | 451         | 24   | 0.133                      | 0.021                       | Dew and Kennedy 1980  |
| Mustelidae                     |             |      |                            |                             |                       |
| <i>Mustela erminea</i>         | 39          | 21   | 0.000                      | 0.000                       | Simonsen 1982         |
| <i>Mustela nivalis</i>         | 13          | 21   | 0.000                      | 0.000                       | Simonsen 1982         |
| <i>Mustela putorius</i>        | 24          | 21   | 0.000                      | 0.000                       | Simonsen 1982         |
| <i>Martes martes</i>           | 2           | 21   | 0.000                      | 0.000                       | Simonsen 1982         |
| <i>Martes foina</i>            | 121         | 21   | 0.000                      | 0.000                       | Simonsen 1982         |
| <i>Meles meles</i>             | 5           | 21   | 0.000                      | 0.000                       | Simonsen 1982         |
| Felidae                        |             |      |                            |                             |                       |
| <i>Felis catus</i>             | 56          | 55   | 0.220                      | 0.069                       | O'Brien 1980          |
| <i>Aciononyx jubatus</i>       | 50          | 47   | 0.000                      | 0.000                       | O'Brien et al. 1983   |
| Herpestidae                    |             |      |                            |                             |                       |
| <i>Herpestes auropunctatus</i> | 45          | 29   | 0.241                      | 0.037                       | unpublished data      |
|                                |             |      | $\bar{x} = 0.062$          | $\bar{x} = 0.014$           |                       |

\*Same population as above, including analysis of 16 additional loci.

If the data for humans and *Peromyscus* are typical, these three loci are highly variable and tend to give a higher estimation of genetic variation than estimates based on a larger number of loci. This would suggest that a great deal of genetic variation has been lost from the Meeteetse population of BFFs. However, the estimates of genetic variation in these taxa are based, for the most part, on reports of genetic variation at a single locus and not on surveys of a number of loci. This would appear to result in a biased data set, since loci observed to be monomorphic (invariable) are not reported unless they are part of a survey of loci.

If the data for the small Indian mongoose, *Herpestes auropunctatus*, (Table 1) are typical (or typical for carnivores), these loci demonstrate little or no genetic variation. This would suggest that these loci provide little information concerning the total levels of genetic variation present in the Meeteetse BFF population. The comparative data avail-

able concerning the genetic variation at these three loci (Table 1) are inconclusive for providing a meaningful interpretation of the observed absence of genetic variation at these loci in the BFF.

Although it is important to continue attempts to determine the existence of genetic variability that could be managed in the Meeteetse BFF population, the absence of genetic variation observed thus far may be the result of a recent bottleneck or may be typical for carnivores. Some mammals that have passed through severe bottlenecks demonstrate an absence of or a very low level of heterozygosity (Bonnell and Selander 1974, Ryman et al. 1977, O'Brien et al. 1983). However, the North American bison (*Bison bison*), which has also gone through a bottleneck, has a mean heterozygosity of 0.023, and a small Indian mongoose population, which was derived from a few individuals introduced to St. Croix in 1884, presently has a level of heterozygosity of 0.037. The effect of the bot-

tleneck on the level of genetic variability is dependent upon the rate at which the population recovers from the reduced population size (Smith 1981) and not on the bottleneck alone.

Pettus (1985) suggested that carnivores and perhaps other species of large mammals are employing the Mullerian strategy and would be expected to exhibit little genetic variation. Carnivores appear to have somewhat lower levels of genetic variation (Table 2), with a mean heterozygosity of 0.014 for 16 species as compared to a mean heterozygosity for 46 species of mammals of 0.036 (Nevo 1978). No genetic variation has been observed in any of the six taxa of the family Mustelidae (Table 2) that have been examined. Unfortunately, these have been examined by only one laboratory (Simonsen 1982), and the sample sizes of some taxa were very small.

Although the mean heterozygosity observed in carnivores is below the mean value of other mammalian taxa, several species demonstrate levels of heterozygosity typical for mammals (Table 2). Those taxa that demonstrated the highest levels of genetic variation among carnivores (Table 2), *Felis catus* and *Canis latrans*, are those with estimates based on the largest number of loci. The effect of examining a small number of loci is clearly seen in the estimates of genetic variation in the American black bear (*Ursus americanus*), as pointed out by Manlove et al. (1980). The level of genetic variability in different populations increases with the number of loci examined (Table 2).

Future work should include continued research to provide an estimate of genetic variation in the Meeteetse population of BFF based on a larger number of loci. This research could include an examination of additional loci from nontraditional sources such as saliva (Tan and Teng 1979), urine (Hayakawa et al. 1983), and feces (Scribner and Warren 1984). Examination of blood samples (hemolysate and serum), however, would allow detection of genetic variation at 30 to 40 loci.

By including loci for proteins from nontraditional sources, other surveys of genetic variation in mammal taxa could provide a better understanding of levels of genetic variation present at these loci. Other surveys of genetic

variation in carnivores, especially within the mustelids, including loci for proteins from traditional and nontraditional sources, would provide a better data base from which to determine what portion of the total genetic variability could be expected to be identified from salivary samples.

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